

## Using flow cytometry to compare the dynamics of photoreceptor outer segment phagocytosis in iPS derived RPE cells.

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### Public Summary:

Light entering the eye activates photoreceptor neurons that convert the stimulus into electrical impulses. These impulses are passed back through the eye and into the brain where they form the basis of vision. The rod and cone photoreceptors are highly dependent on other retinal cell types to function properly. Retinal pigment epithelium (RPE) cells are especially important; these protect the photoreceptors from light-induced toxicity by consuming the tips of the photoreceptor cells that are routinely damaged by intense light exposure. RPE cells perform this essential task (named phagocytosis) on a daily basis. If RPE cells die or become dysfunctional, i.e. by not effectively performing phagocytosis, photoreceptors will invariably die. This phenomenon occurs in human diseases including age-related macular degeneration (AMD), the leading cause of vision loss in the elderly. A promising potential therapy is to implant RPE generated from stem cells into the back of the eye to replace diseased patient cells. Several groups, including our own, have shown that this therapy works very well in rats with spontaneous retinal degeneration, but several technical questions remain about how well the implanted cells function compared with actual RPE cells. Since phagocytosis is such a critical function we developed a unique way to test how well RPE cells can phagocytose the tips of the photoreceptor cells before implantation. We utilized a method named flow cytometry that is used to measure fluorescence in single cells. RPE cells can become fluorescent if they are "fed" photoreceptor outer segments that are treated with green fluorescent biomarkers. We waited several hours after feeding the cells and then measured the amount of green fluorescence they were emitting. In this study we were able to demonstrate that RPE cells we generated from stem cells chronologically phagocytosed equal numbers of photoreceptor outer segments as well as actual human RPE cells do. We also showed using flow cytometry that the stem cell derived RPE generated the correct cellular machinery required for efficient phagocytosis. We suggest, therefore, that this technique be employed in the field to determine if stem cell derived RPE cells are ready for transplantation. The results of this study add to mounting evidence that stem cell derived RPE function as well as real RPE cells, and are realistic therapeutic options for treating AMD.

### Scientific Abstract:

**Purpose:** Retinal pigment epithelium (RPE) autologous grafts can be readily derived from iPS cells (iPS-RPE). It is critical to stringently characterize iPS-RPE using standardized and quantifiable methods to be confident that they are safe and adequate replacements for diseased RPE before utilizing them in clinical settings. One important and required function is that the iPS-RPE phagocytose photoreceptor outer segments (POS). **Methods:** We developed a flow cytometry-based assay to monitor binding and internalization of FITC labeled POS by ARPE-19, human fetal RPE (hFRPE), and two types of iPS-RPE. Expression and density of  $\alpha$ 5 $\beta$ 1 integrin, CD36, and MerTK receptors, which are required for phagocytosis, were compared. **Results:** Trypsinization of treated RPE cells results in the release of bound POS. The number of freed POS, the percentage of cells that internalized POS, the brightness of the FITC signal from the cells, and the surface density of the phagocytosis receptors on single RPE cells was measured using flow cytometry. These assays reveal that receptor density is dynamic during differentiation and this can affect the binding and internalization dynamics of the RPE cells. Highly differentiated iPS-RPE phagocytose POS more efficiently than hFRPE. **Conclusions:** Caution should be exercised to not use RPE grafts until demonstrating that they are fully functional. The density of the phagocytosis receptors is dynamic and may be used as a predictor for how well the iPS-RPE cells will function in vivo. The phagocytosis dynamics observed between iPS-RPE and primary RPE is very encouraging and adds to mounting evidence that iPS-RPE may be a viable replacement for dysfunctional or dying RPE in human patients.

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